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Louchami, Karim ; Best, Len ; Brown, Peter ; Virreira, Myrna ; Hupkens, Emeline ; Perret, Jason ; Devuyst, Olivier ; Uchida, Shinichi ; Delporte, Christine ; Malaisse, Willy J ; Beauwens, Renaud ; Sener, Abdullah

Abstract: UNLABELLED: BACGROUNDS/AIMS: Several insulinotropic agents were recently reported to cause β -cell swelling. The possible participation of AQP7 to water transport was investigated in AQP7(+/+) or AQP7(-/-) mice. **METHODS:** Aquaporin expression, insulin secretion, cell swelling and electrical activity were investigated in pancreatic islets. **RESULTS:** RT-PCR revealed the expression of AQP5 and AQP8 mRNA. Double immunofluorescent labeling indicated their presence in β -cells. Whilst basal insulin release from isolated pancreatic islets incubated at 2.8 mM D-glucose did not differ between AQP7(+/+) or AQP7(-/-) mice, the secretion of insulin evoked by the omission of 50 mM NaCl, the substitution of 50 mM NaCl by 100 mM glycerol or a rise in D-glucose concentration to 8.3 mM and 16.7 mM was severely impaired in the islets from AQP7(-/-) mice. Yet, exposure of β -cells to either the hypotonic medium or a rise in D-glucose concentration caused a similar degree of swelling and comparable pattern of electrical activity in cells from AQP7(+/+) and AQP7(-/-) mice. Both the cell swelling and change in membrane potential were only impaired in AQP7(-/-) cells when exposed to 50 mM glycerol. **CONCLUSION:** It is proposed, therefore, that AQP7 may, directly or indirectly, play a role at a distal site in the exocytotic pathway.

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A New Role for Aquaporin 7 in Insulin Secretion

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Abstract

Background/Aims: Several insulinotropic agents were recently reported to cause β -cell swelling. The possible participation of AQP7 to water transport was investigated in AQP7^{+/+} or AQP7^{-/-} mice. **Methods:** Aquaporin expression, insulin secretion, cell swelling and electrical activity were investigated in pancreatic islets. **Results:** RT-PCR revealed the expression of AQP5 and AQP8 mRNA. Double immunofluorescent labeling indicated their presence in β -cells. Whilst basal insulin release from isolated pancreatic islets incubated at 2.8 mM D-glucose did not differ between AQP7^{+/+} or AQP7^{-/-} mice, the secretion of insulin evoked by the omission of 50 mM NaCl, the substitution of 50 mM NaCl by 100 mM glycerol or a rise in D-glucose concentration to 8.3 mM and 16.7 mM was severely impaired in the islets from AQP7^{-/-} mice. Yet, exposure of β -cells to either the hypotonic medium or a rise in D-glucose concentration caused a similar degree of swelling and comparable pattern of electrical activity in cells from AQP7^{+/+} and AQP7^{-/-} mice. Both the cell swelling and change in membrane potential were only impaired in AQP7^{-/-} cells when exposed to 50 mM glycerol. **Conclusion:** It is proposed, therefore, that AQP7 may, directly or indirectly, play a role at a distal site in the exocytotic pathway.

Key Words

AQP7 • Insulin-producing β -cells • Insulin secretion • Cell swelling

Introduction

Aquaporins are channel-forming membrane proteins which allow water movement through the plasma membrane [1]. Aquaglyceroporins represent a subfamily of aquaporins permeable not only to water but also to small solutes like glycerol and urea [1, 2]. Aquaglyceroporin 7 (AQP7) is expressed in rat and mouse pancreatic islet β -cells and tumoral insulin-producing BRIN-BD11 cells [3-5]. Matsumura et al. [5] first reported that islets isolated from AQP7^{-/-} mice secrete more insulin than islets obtained from AQP7^{+/+} mice, when incubated at either low (5.6 mM) or high (25.0 mM) Dglucose concentration, despite a lower islet insulin content

in the former AQP7^{-/-} mice than in the latter AQP7^{+/+} mice. Incidentally and quite surprisingly, assuming an islet protein content close to 1.0 µg/islet [6], the secretion of insulin by islets from AQP7^{+/+} mice recorded in the presence of 25.0 mM D-glucose was about two orders of magnitude lower (ca. 23.8 ± 1.5 pg/µg protein per hour; n = 3) in the study by Matsumura et al. [5] than that found either by Li et al. [7] in islets from wild-type mice incubated in the presence of 20.0 mM D-glucose (about 2.0 ± 0.1 ng/µg protein per hour; n = 9-23) or by Bulur et al. [8] in islets from NMRI mice incubated at 16.7 mM D-glucose (3.8 ± 0.5 ng/µg protein per hour). An even more pronounced relative difference (about 400-fold) prevails when comparing the insulin content from wildtype mice in the report by Matsumura et al. (113.4 ± 7.2 pg/µg protein; n = 8) and either Li et al. (47 ± 3 ng/µg protein; n = 6-8) or Bulur et al. (46 ± 1 ng/µg protein; n = 88). Matsumura et al. [5] also observed increased intraislet glycerol and triglyceride contents in the AQP7^{-/-} mice compared to those in the AQP7^{+/+} mice. They concluded that AQP7 appears to be a key regulator of intracellular glycerol content as well as insulin production and secretion.

Another possible role for aquaporins in the process of nutrient-stimulated insulin secretion is currently considered in the framework of the so-called volumeregulated anion channel hypothesis [9, 10]. It postulates that, in addition to metabolically-regulated K_{ATP} channels, β-cells are equipped with volume-regulated anion channels (VRAC) that are activated by D-glucose concentrations within the range effective in modulating electrical activity and insulin release. More precisely, it is proposed that the intracellular accumulation of lactate and bicarbonate anions generated in insulin-producing cells by the catabolism of D-glucose leads to cell swelling and, hence, gating of the volume-sensitive anion channels, eventually leading to plasma membrane depolarization and subsequent gating of voltage-dependent calcium channels [11]. Such a hypothesis motivated recent investigations in the perspective of the possible participation of AQP7 to the regulation of insulin secretion in pancreatic β-cells. For instance, it was recently demonstrated that glycerol depolarizes pancreatic β-cells, resulting in electrical and secretory activity [3]. Similar effects were observed using a non-metabolizable glycerol analogue, 1,3-propanediol, and with urea. In common with glycerol, these osmolytes permeate the β-cell plasma membrane, probably via AQP7. In fair agreement with the latter proposal, the net uptake of [2-³H]glycerol by BRIN-BD11 cells increased to $143 \pm 4\%$ of its control value within 2 min incubation in a hypotonic extracellular medium and progressively declined thereafter, both the relative magnitude and time course of such changes being similar to those observed under the same experimental conditions for the initial increase in cell volume and later regulatory volume decrease [4,12].

Based on a comparison between AQP7^{+/+} wild-type mice and AQP7^{-/-} knockout mice, this study further investigates the role of AQP7 in pancreatic β -cell function. Taking also into account novel information on the expression of AQP5 and AQP8 in insulin-producing cells, the present report proposes a new role for AQP7 in insulin secretion.

Materials and Methods

Animals

AQP7^{-/-} mice were generated at Tokyo Medical and Dental University by targeted deletion of exon 2 [13] and sent to Belgium, where they were maintained in air-filtered cages and fed normal mouse chow in the Division of Nephrology of the Université Catholique de Louvain. Body weight, as well as plasma D-glucose [14] and insulin [15] concentration were measured in male and female AQP7^{+/+} and AQP7^{-/-} mice by methods described in the cited references.

Islet isolation

Pancreatic islets were isolated by the collagenase procedure [16].

RT-PCR

Total RNA from mouse tissues was extracted using the AURUMTM total RNA fatty acid and tissue kit (BioRad, Hercules, CA, USA). The purified total RNA was quantified on a Nanodrop spectrophotometer (NanoDrop Technologies, Inc, Wilmington, DE, USA). Quality of the total RNA was verified on an Experion Automated Electrophoresis System (BioRad, Hercules, CA, USA). The total RNA was reverse transcribed using the Revert AidTM first strand cDNA synthesis kit (Fermentas, St. Leon-Rot, Germany), starting from 1 μ g total RNA in a final 20 μ l reaction volume. The sequences of forward and reverse mouse AQPs and beta actin are shown in Table 1. All PCR reactions were performed in a total volume of 20 μ l containing 1 μ l of cDNA, 0.5 U GoTaq DNA polymerase (Promega, Madison, WI, USA), 0.2 mM dNTP, 0.5 μ M of each primer, and 4 μ l GoTaq Green 5x buffer using My iCycler Thermal cycler (Bio-Rad Laboratories, Hercules, USA). Thermocycling conditions were 94°C for 1.5 min followed by 35 cycles of 30 sec at 95°C, 30 sec at 60°C and 1 min at 72°C. The PCR amplification products were submitted to electrophoresis on a 1.2% agarose gel in TAE buffer: 40 mM Tris, 40 mM Acetate, 1 mM EDTA in the presence of 0.5 μ g/ml of ethidium bromide. Gels were visualized by UV transillumination using a GelDoc apparatus (Bio-Rad Laboratories, Hercules, USA).

Double fluorescence immunolabelling

Several sections of mouse pancreas, embedded in paraffin (4 μ m thickness), were deparaffined, rehydrated and then incubated overnight at 4°C with primary antibodies as follows: rabbit polyclonal affinity purified anti-AQP5 (Millipore, Billerica, MA, USA) or anti-AQP8 (Alpha Diagnostics, San Antonio, TX, USA) at dilution 1:100 and mouse monoclonal affinitypurified anti-insulin at dilution 1:100 (Abcam, Cambridge, United Kingdom). Bound antibodies were revealed by incubating the tissue slices with anti-rabbit biotin antibodies at a dilution of 1:200 in PBS containing 5% horse serum (GE Healthcare, Little Chalfont, United Kingdom) for 120 min. This was followed by incubation with streptavidin-Cyanin2 (dilution 1:300 in PBS containing 5% horse serum (GE Healthcare, Little Chalfont, United Kingdom)) and anti-mouse-Cyanin3 (dilution 1:300 in PBS containing 5% horse serum (GE Healthcare, Little Chalfont, United Kingdom) for 120 min. Slides were mounted using Pro-Long Gold antifade reagent (Invitrogen, Eugene, USA). As

negative controls, tissue sections were incubated either with secondary antibody alone, or with anti-AQP antibody preadsorbed with a 100-fold excess of the immunizing peptide.

Images were taken using an Axiocam MRB fluorescent microscope using a 40x objective.

Insulin secretion

Insulin secretion was measured in groups of 8 islets incubated for two successive periods of 30 min each in 1.0 ml of salt-balanced medium [16] containing 5.0 mg/ml bovine albumin and equilibrated against a mixture of O₂-CO₂ (95-5, vv).

Cell volume and electrical activity

Cell volume measurements and electrophysiological recordings were carried out on isolated mouse β -cells. For this purpose, islets were dispersed by brief exposure to Ca²⁺-free medium containing NaCl (130 mM), KCl (5 mM), MgCl₂ (1 mM), D-glucose (5 mM) EGTA (1 mM) and HEPES-NaOH (20 mM; pH 7.4). Islet cells were centrifuged at 500g for 5 min, resuspended in HEPES-buffered Minimal Essential Medium

(Invitrogen, Paisley, UK) supplemented with 5% (v/v) foetal calf serum and 50 μ g/ml gentamicin and cultured in 30 mm diameter polystyrene dishes for up to one week in humidified air at 37°C. Relative cell volume (RCV) was measured using a video-imaging technique as described previously [3, 17]. β -cells were distinguished from other islet cells by size and characteristic granular appearance. The mean volume (\pm SEM) of the cells used in this study was 3.3 ± 0.2 pl ($n = 45$; range 1.7 to 5.7 pl). Two series of experiments were performed. In the first the effects of superfusing cells with a hypotonic solution were examined. The isotonic solution (329 ± 1 mOsmol.Kg H₂O⁻¹, $n = 6$) contained NaCl (139 mM), KCl (5 mM), NaHCO₃ (25 mM), D-glucose (2.8 mM), mannitol (13.9 mM), MgCl₂ (1.2 mM), CaCl₂ (1 mM) with a pH 7.4 and was gassed with 95% O₂ and 5% CO₂. The hypotonic solution (236 ± 1 mOsmol.Kg H₂O⁻¹, $n = 5$) had a similar composition but contained only 89 mM NaCl and no mannitol. The second series of experiments examined volume changes in response to solutions containing either an increased glucose concentration or glycerol. The solutions used in these experiments were similar to those employed in previous studies examining the effects of D-glucose [17] and glycerol [3] on cell volume. Thus, in the D-glucose experiments the solutions (pH 7.4) contained NaCl (110 mM), KCl (5 mM), NaHCO₃ (25 mM), MgCl₂ (1.2 mM), CaCl₂ (1 mM) and were gassed with 95% O₂ and 5% CO₂. The D-glucose concentrations were either 4 or 20 mM and osmolality was maintained at 290 ± 2 mOsmol.Kg H₂O⁻¹, $n = 3$) by the addition of 16 mM mannitol to the 4 mM D-glucose solution. Hepes-buffered solutions were used in the glycerol experiments, and these contained NaCl (120 mM), KCl (5 mM), D-glucose (4 mM), MgCl₂ (1.2 mM), CaCl₂ (1 mM), Hepes/NaOH (5 mM; pH 7.4) and either 50 mM mannitol (309 ± 0 mOsmol.Kg H₂O⁻¹, $n = 3$) or 50 mM glycerol (307 ± 1 mOsmol.Kg H₂O⁻¹, $n = 3$). Cells were superfused with these solutions at a rate of 5.5 ml.min⁻¹ and at a temperature of 37°C. Images were saved as JPEG files at a rate of 1 per minute, except in one series of experiments in which the rate of cell swelling in the hypotonic solution was recorded at one image every 15 seconds. Cell volume was calculated from the area of each image assuming the cells are spherical. Volumes were normalised to the volume observed during the 2 min control period at the beginning of each experiment (relative cell volume). Cells were equilibrated with the experimental solution for 8 min before each experiment. Rates of volume change were calculated by linear regression analysis of the linear phase (a minimum of 4 data points were employed in these calculations). Volume changes in response to glucose and glycerol were calculated using the area under the curve function of Graphpad 5 (Prism software).

®-cell electrical activity

The electrical activity of ®-cells was recorded by means of the perforated patch technique using a List EPC-7 amplifier in current clamp mode as described previously [18]. Cells were superfused with a bath solution consisting of NaCl (130 mM), KCl (4 mM), MgCl₂ (1.2 mM), CaCl₂ (1 mM), D-glucose (2.8 mM) and HEPES-NaOH (20 mM; pH 7.4). For experiments studying the effects of glycerol, the basal medium contained 50 mM mannitol substituted for 25 mM NaCl. The addition of glycerol to the medium was then substituted for an equivalent amount of mannitol. The pipette solution contained KCl (130 mM), NaCl (4 mM), MgCl₂ (1 mM), HEPES-NaOH (10 mM; pH 7.2) and gramicidin D (50 µg/ml) as perforating agent. Activity of the volume-regulated anion channel (VRAC) was measured using the conventional whole-cell recording technique [19]. Cell swelling was induced by use of a hypertonic pipette solution consisting of CsCl (60 mM), MgCl₂ (2 mM), ATP (1 mM), EGTA (1 mM), mannitol (220 mM) and HEPES (10 mM, pH 7.2). CsCl (1 mM) was added to the bath solution to block inward K⁺ currents. Cells were held at 0 mV and subjected to 50 msec voltage pulses of ± 100 mV at 2 sec intervals. Cell capacitance (7–11 pF) was measured by nulling the capacitance transients, and current density expressed as pA/pF.

Statistical analysis

All results are expressed as means \pm SEM. The statistical significance of differences between mean values was assessed by use of Student's *t*-test.

Results

Body weight, plasma D-glucose and insulin concentrations

As shown in Table 2, both the AQP7^{+/+} and AQP7^{-/-} female mice displayed a lower body weight ($p < 0.05$ or less) than the corresponding male mice. No significant difference was observed between the four groups of mice listed in Table 2, in terms of either plasma D-glucose or insulin concentration.

Aquaporin expression in rat pancreatic islet cells

Screening of AQP5 mRNA expression in mouse pancreatic islet cells was performed by RT-PCR, using ®-actin primers or mouse AQP5 specific primers on cDNA obtained from purified total RNA isolated from several mouse tissues (used as positive controls) and mouse pancreatic islet cells. The amplification reaction yielded single amplicon of the expected molecular weight for ®-actin, AQP5 and AQP8 in pancreatic islet cells from both AQP7^{+/+} and AQP7^{-/-} mice (Fig. 1), whilst such was only the case for AQP7 in islet cells from AQP7^{+/+} mice as distinct from AQP7^{-/-} mice.

Double immunofluorescence labeling of AQPs and insulin in mouse pancreatic sections indicated that AQP5 and AQP8 were expressed by insulin-producing ®-cells (Fig. 2).

Insulin secretion

In a first series of experiments, the release of insulin over 30 min preincubation in an isotonic medium containing 2.8 mM D-glucose did not differ significantly ($p > 0.5$) from the islets of either AQP7^{+/+} mice (12.0 ± 1.1 µU/

Table 2. Body weight, plasma D-glucose and insulin concentrations.

islet; $n = 60$) or AQP7^{-/-} mice ($13.0 \pm 1.3 \mu\text{U}/\text{islet}$; $n = 60$). When further incubated for 30 min under the same experimental conditions, the release of insulin, expressed relative to the paired value recorded during preincubation, again failed to differ significantly ($p = 0.95$) in the WT animals ($50.7 \pm 4.7\%$; $n = 20$) and knock-out mice ($50.1 \pm 8.3\%$; $n = 20$). When the final incubation was conducted at the low D-glucose concentration (2.8 mM) in a medium of low osmolarity, as provoked by the omission of 50 mM NaCl, the paired incubation/preincubation ratio for insulin release averaged, in the control animals, $204.8 \pm 54.2\%$ ($n = 20$), a value 4-fold higher ($p < 0.008$) than that recorded when both the preincubation and incubation were conducted in an isotonic medium (Fig. 3). In the knockout mice, however, the paired incubation/preincubation ratio after exposure to the hypotonic medium did not exceed ($61.6 \pm 6.6\%$; $n = 18$), a value no more significantly different ($p > 0.29$) from that recorded, in the same mice, when both the preincubation and incubation were conducted in an isotonic medium. When the final incubation medium was conducted in an isotonic medium, in which 50 mM NaCl was substituted by 100 mM glycerol, the incubation/preincubation ratio for insulin release averaged, in the control animals, $91.6 \pm 13.9\%$ ($n = 20$) a value again significantly higher ($p < 0.009$) from that recorded when both the preincubation and incubation were conducted in an isotonic medium. In the knock-out mice, the incubation/preincubation ratio for insulin output after exposure to the isotonic medium with substitution of NaCl (50 mM) by glycerol (100 mM) did not exceed $54.7 \pm 7.7\%$ ($n = 17$) and, as such, failed to differ significantly ($p > 0.69$) from that recorded in the same mice when both the preincubation and incubation were conducted in the control isotonic medium.

In a second series of experiments, the islets from control and knock-out mice were again preincubated for 30 min in a salt-balanced medium containing 2.8 mM D-glucose and then incubated in the same medium containing either 2.8, 8.3 or 16.7 mM D-glucose (Fig. 4). During the preincubation, the release of insulin was not significantly different ($p > 0.18$) in the islets from control mice ($24.0 \pm 2.9 \mu\text{U}/\text{islet}$; $n = 52$) and knock-out mice ($19.9 \pm 1.6 \mu\text{U}/\text{islet}$; $n = 71$). When the islets were further incubated at 2.8 mM D-glucose, the release of insulin expressed relative to the paired value recorded during preincubation also failed to differ significantly ($p > 0.39$) in the control mice ($44.8 \pm 5.7\%$; $n = 17$) and knock-out mice ($38.3 \pm 5.1\%$; $n = 21$). However, when the islets were eventually incubated at 8.3 mM D-glucose, the paired incubation/preincubation ratio for insulin output was lower ($p < 0.04$) in the knock-out mice ($185.5 \pm 29.6\%$; $n = 23$) than in the control mice ($323.9 \pm 64.2\%$; $n = 15$). The latter two percentages were both much higher ($p < 0.001$) than those recorded in the same type of mice (control or knock-out mice) when the islets were eventually exposed to only

2.8 mM. Likewise, when the islets were eventually incubated at 16.7 mM D-glucose, the paired incubation/preincubation ratio for insulin output was lower ($p < 0.001$) in the knock-out mice ($253.9 \pm 48.4\%$; $n = 23$) than in the control mice ($676.2 \pm 89.8\%$; $n = 16$).

The unfavourable effect of the absence of AQP7 affected to the same relative extent the secretory response to distinct secretagogues. Indeed, the incubation/preincubation ratio for insulin release from the islets of knock-out mice averaged, in the first series of experiments, i.e. in islets eventually incubated at 2.8 mM D-glucose in media deprived of 50 mM NaCl, whether in the absence or presence of 100 mM glycerol, $51.7 \pm 6.2\%$ ($n = 35$) of the corresponding reference values found within the same experiments and under the same experimental conditions in the AQP7^{+/+} mice ($100.0 \pm 12.2\%$; $n = 40$), the former value not being significantly different ($p > 0.45$) from that recorded in the second series of experiments, i.e. in islets eventually exposed to 8.3 or 16.7 mM D-glucose, in which case the mean value found in the AQP7^{-/-} mice averaged $46.3 \pm 5.5\%$ ($n = 46$) of the corresponding reference values ($100.0 \pm 9.8\%$; $n = 31$). In other terms, the relative magnitude of the decrease in the secretory response of islets from AQP7^{-/-} mice was virtually identical ($p > 0.75$) in the first ($48.3 \pm 14.2\%$; $df = 73$) and second ($53.6 \pm 10.5\%$; $df = 75$) set of experiments.

Cell volume changes in response to extracellular hypotonicity, D-glucose or glycerol

Fig. 5A shows volume changes in mouse β -cells superfused with hypotonic solutions. When exposed to hypotonic solution the cells from AQP7^{+/+} animals swelled reaching a maximum relative cell volume of 1.23 ± 0.01 ($n = 7$) within 3 to 4 min (Fig. 5A, squares). The cells then exhibited a regulatory volume decrease (RVD) to 1.12 ± 0.01 by the end of the hypotonic period. The response of the β -cells from AQP7^{-/-} mice (Fig. 5A, circles) was almost identical to that of cells from the AQP7^{+/+} animals. Thus cell volume increased to a maximum of 1.24 ± 0.01 ($n=4$; $p > 0.1$), and exhibited a RVD which was similar to the ^{+/+} cells, i.e. the initial rate of decrease was -0.016 ± 0.001 RCV.min⁻¹ in both populations ($p > 0.1$), and the final volume was 1.14 ± 0.01 in the AQP7^{-/-} cells which is not different to AQP7^{+/+} ($p > 0.1$). Fig. 5B shows the rate of the initial volume changes in response to the hypotonic solution with a resolution of 15 seconds. There is no difference in the rate of volume change between six cells from AQP7^{+/+} mice and six cells from ^{-/-} animals (0.092 ± 0.011 versus 0.075 ± 0.010 , $p > 0.1$).

In six AQP7^{+/+} cells exposed to an increase in D-glucose concentration from 4 to 20 mM, the volume increased to a maximum of 1.06 ± 0.01 during a 10 min exposure to 20 mM D-glucose (Fig. 6A, squares). Similar increases in cell volume were observed in β -cells from ^{-/-} mice ($n = 6$). Thus the maximum volume was

1.06 \pm 0.01 (not different to wild-type, $p > 0.1$). Furthermore the volume changes (measured as the area under the curve different to 1.0), were not different, being 0.35 \pm 0.10 RCV.min in +/+ and 0.32 \pm 0.09 RCV.min in -/- ($p > 0.1$). @-cell volume also increased when cells were exposed to 50 mM glycerol (Fig. 6B). However, the volume change in AQP7^{-/-} cells (0.49 \pm 0.11 RCV.min, $n = 6$) was significantly lower than that observed in AQP7^{+/+} cells (volume change = 1.02 \pm 0.16 RCV.min; $p < 0.02$). Incidentally, at variance with the response to hypotonicity (Fig. 5), that to either a rise in D-glucose concentration or the substitution of mannitol by glycerol (Fig. 6) failed to display a regulatory volume decrease.

Electrophysiological data

Electrical responses of @-cells to various stimuli are shown in Fig. 7 and 8. In the AQP7^{+/+} @-cells, a rise in D-glucose concentration from 2.8 mM to 8.3, 11.1 or 16.7 mM provoked a depolarization of the plasma membrane leading to the generation of action potentials (Fig. 7). Exposure of the @-cells from AQP7^{+/+} mice to a 33% hypotonic medium, to 50 μ M tolbutamide or to 50 mM glycerol (substituted for an equal concentration of mannitol) also caused plasma membrane depolarization and electrical activity (Fig. 8). Essentially comparable results were recorded in @-cells from AQP7^{-/-} mice when exposed to a rise in D-glucose concentration up to 8.3, 11.1 or 16.7 mM (Fig. 7), to the 33% hypotonic medium or to 50 μ M tolbutamide (Fig. 8, upper traces). However, in sharp contrast to the @-cells from AQP7^{+/+} mice, cells from AQP7^{-/-} mice failed to display any significant change in plasma membrane potential when exposed to 50 mM glycerol (Fig. 8, lower traces). Thus, in the experiments concerning the response to glycerol, the resting membrane potential in the presence of 2.8 mM D-glucose was virtually identical in @-cells from AQP7^{+/+} (- 65.5 \pm 2.3 mV; $n = 6$) and AQP7^{-/-} (- 67.0 \pm 1.2 mV; $n = 4$). In cells from AQP7^{+/+} mice, addition of glycerol caused a significant ($p < 0.05$) change in membrane potential to - 53.8 \pm 3.7 mV ($n = 6$). However, in AQP7^{-/-} cells, the corresponding value during exposure to glycerol was - 66.8 \pm 0.8 mV ($n = 4$, $p > 0.05$).

The last series of experiments investigated activity of the volume-regulated anion channel (VRAC) in @-cells from AQP7^{+/+} and -AQP7^{-/-} mice (Fig. 9). In both cases, channel activation was induced by swelling the cells using a hypertonic intracellular solution under conventional whole-cell recording conditions. In AQP7^{+/+} @-cells, maximal outward (I_o) and inward (I_i) currents averaged, respectively, +99.5 \pm 6.8 and -26.0 \pm 3.2 pA/pF ($n = 10$). Comparable values were recorded from AQP7^{-/-} cells, i.e. respectively +112.2 \pm 13.9 and - 32.2 \pm 4.3 pA/pF ($n = 9$). The rate of activation of the VRAC current was also comparable in the AQP7^{+/+} and AQP7^{-/-} cells, halfmaximal activation ($T_{0.5}$) being achieved, respectively, 120 \pm 25 ($n = 9$) and 173 \pm 43 ($n = 9$) seconds following rupture

of the membrane patch. The reversal potential of the VRAC current (V_{rev}) was -13.0 ± 0.4 mV ($n = 5$) and -10.3 ± 0.8 mV ($n = 5$) in the $+/+$ and $-/-$ cells respectively. Thus, no significant differences in VRAC kinetics were apparent between AQP7 $+/+$ and AQP7 $-/-$ β -cells.

Discussion

The present study reveals that the release of insulin evoked by a rise in D-glucose concentration, by extracellular hypotonicity or by the isosmotic addition of glycerol in isolated pancreatic islets was always lower in AQP7 $-/-$ than in AQP7 $+/+$ mice. These findings contrast with those reported by Matsumura et al. [5] who observed, at both low (5.6 mM) and high (25.0 mM) D-glucose concentrations, a higher insulin output from islets isolated from AQP7 $-/-$, as distinct from AQP7 $+/+$, mice, despite a lower islet insulin content in the former AQP7 $-/-$ mice than in the latter AQP7 $+/+$ mice. In considering such conflicting findings, it should be emphasized that all secretory data in the present study were analyzed by paired comparison with the basal insulin output recorded over 30 min preincubation in an isotonic medium containing 2.8 mM D-glucose. Pooling together all available data, the basal insulin output averaged 17.6 ± 1.6 ($n = 112$) and 16.7 ± 1.1 μ U/islet ($n = 131$) in AQP7 $+/+$ and AQP7 $-/-$ mice respectively, leaving little doubt as to the lack of any statistically significant difference ($p > 0.66$) between these two mean values. In terms of volume regulation, isolated β -cells from AQP $+/+$ and AQP7 $-/-$ mice both showed comparable rates and degrees of cell swelling when exposed to a hypotonic solution. This finding implies the existence of at least one water transport pathway in mouse β -cells in addition to AQP7. In this regard, Matsumura and colleagues [5] noted the expression of AQP8 in mouse β -cells, whilst the Na/K/2Cl transporter NKCC1, also expressed in β -cells [20], has been reported to act as a water co-transporter [21]. The present study confirms the expression of AQP8 and reveals that of AQP5 in mouse β -cells. β -cells from AQP $+/+$ and AQP7 $-/-$ cells also showed similar increases in volume in response to a rise in D-glucose concentration, an effect previously attributed to the intracellular accumulation of D-glucose metabolites [17]. This finding suggests that D-glucose metabolism is not significantly impaired in AQP7 $-/-$ β -cells. In contrast, a significant difference was observed in the volume responses of AQP $+/+$ and AQP7 $-/-$ cells to glycerol. Thus, the isosmotic addition of glycerol (substituting for mannitol) caused a significantly smaller increase in cell volume in the AQP7 $-/-$ cells compared to those from wild type mice, presumably reflecting the activity of AQP7 as a glycerol transport pathway. This finding is consistent with the previous suggestion that AQP7 mediates both the influx [4] and efflux [5] of glycerol from insulin-producing cells. It should, however, be noted that the residual degree of swelling in AQP7 $-/-$ cells in response to glycerol implies the existence of an additional, as yet unidentified, pathway

of glycerol transport.

The recordings of β -cell electrical activity in the present study were consistent with cell volume changes. Thus, a rise in glucose to stimulatory concentrations was routinely found to cause depolarization and generate electrical activity in cells from both AQP $^{+/+}$ and AQP7 $^{-/-}$ mice. Similarly, exposure to hypotonic solutions was found to cause depolarization in wild type and knockout β -cells, presumably reflecting swelling-induced VRAC activation generating an inward current. Virtually identical responses were also seen in these cells to the application of the hypoglycemic sulfonylurea tolbutamide. Whilst sulfonylureas are known to inhibit K(ATP) channel activity [22], there is evidence that these drugs can also cause (beta)-cell swelling and increase VRAC activity [23]. However, whilst the isotonic addition of glycerol caused a marked depolarization and electrical activity in AQP7 $^{+/+}$ cells, little or no response was observed in β -cells from AQP7 $^{-/-}$ mice. Again, this finding underlines the proposed role of AQP7 as a glycerol transport pathway.

In view of the apparently normal volume and electrical responses of AQP7 $^{-/-}$ β -cells to various stimuli (with the notable exception of glycerol), it is interesting to note that secretory activity in these cells was impaired compared with those from wild type mice. In this regard, we should emphasise that the secretory data were obtained using intact islets, whereas cell volume and electrophysiological experiments required the use of isolated β -cells. Nevertheless, it is conceivable that, whilst 'proximal' events in β -cell function (including glucose metabolism, cell volume changes and the regulation of electrical activity) are unaffected by the absence of AQP7, this glyceroaquaporin could play a role at a 'distal' site of the exocytotic pathway. Such a proposal is compatible with the finding that the release of insulin by β -cells from AQP7 $^{-/-}$ mice was affected to a comparable relative extent in response to either nutrient or non-nutrient secretagogues. For example, it might imply the perturbed participation of some cytosolic protein otherwise tightly coupled in functional terms to AQP7. Alternatively, the impaired secretory activity of AQP7 $^{-/-}$ β -cells could be

related to a secondary consequence of AQP7 absence, such as the accumulation of triglyceride previously reported in these cells [5]. Consistent with this latter observation, it was noted during the course of the present study that islet cells from AQP7 $^{-/-}$ mice frequently appeared to contain large vacuoles, possibly representing lipid deposits.

In conclusion, the findings of the present investigations strongly suggest a dual role for AQP7 in the regulation of insulin release. On one hand, they are consistent with a key role for AQP7 in allowing both entry [4] and exit [5] of glycerol across the β -cell plasma membrane. Second, the alteration of the secretory

response to D-glucose or extracellular hypoosmolarity in the β -cells from AQP7^{-/-} mice suggests a direct or indirect role for AQP7 at a distal or downstream site in the stimulus-secretion pathway in pancreatic β -cells. The precise determinant(s) of such a second role remain(s), however, to be elucidated.

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References

- 1 Agre P: Aquaporin water channels. *Angew Chem Int Ed Eng* 2004;43:4278-4290.
- 2 Rojek A, Praetorius J, Frokjaer J, Nielsen S, Fenton RA: A current view of the mammalian aquaglyceroporins. *Annu Rev Physiol* 2008;70:301-327.
- 3 Best L, Brown PD, Yates AP, Perret J, Virreira M, Beauwens R, Malaisse WJ, Sener A, Delporte C: Contrasting effects of glycerol and urea transport on rat pancreatic beta-cell function. *Cell Physiol Biochem* 2009;23:255-264.
- 4 Delporte C, Virreira M, Crutzen R, Louchami K, Sener A, Malaisse WJ, Beauwens R: Functional role of aquaglyceroporin 7 expression in the pancreatic beta-cell line BRIN-BD11. *J Cell Physiol* 2009;221:424-429.
- 5 Matsumura K, Chang BH, Fujimiya M, Chen W, Kulkarni RN, Eguchi Y, Kimura H, Kojima H, Chan L: Aquaporin 7 is a beta-cell protein and regulator of intracellular glycerol content and glycerol kinase activity, beta-cell mass, and insulin production and secretion. *Mol Cell Biol* 2007;27:6026-6037.
- 6 Malaisse WJ, Sener A, Koser M, Herchuelz A: Stimulus-secretion coupling of glucose-induced insulin release. Metabolism of α - and β -D-glucose in isolated islets. *J Biol Chem* 1976;251:5936-5943.
- 7 Li D-Q, Jing X, Salehi A, Collins SC, Hoppa MB, Rosengren AH, Zhang E, Lundquist I, Oloffson CS, Mörgelin M, Eliasson L, Rorsman P, Renström E: Suppression of sulfonylurea- and glucose-induced insulin secretion in vitro and in vivo in mice lacking the chloride transport protein CIC-3. *Cell Metab* 2009;10:309-315.
- 8 Bulur N, Zhang Y, Malaisse WJ, Sener A: Insulin release from isolated pancreatic islets, dispersed islet cells and tumoral insulin producing cells : a reexamination. *Metab Funct Res Diab* 2010;3:20-24.
- 9 Best L, Brown PD, Sener A, Malaisse WJ: Electrical activity in pancreatic islet cells: The VRAC hypothesis. *Islets* 2010;2:59-64.
- 10 Best L, McLaughlin J: Nutrients as regulators of endocrine and neuroendocrine

secretion; in: Taylor P, Winderickx J (eds): Topics in Current Genetics. Springer-Verlag, 2004, vol 7, pp 79-111.

11 Malaisse WJ, Best L, Beauwens R, Sener A: Ionic determinants of the insulinotropic action of glucose: the anion channel hypothesis. *Metab Funct Res Diab* 2008;1:2-6.

12 Beauwens R, Best L, Markadieu N, Crutzen R, Louchami K, Brown P, Yates AP, Malaisse WJ, Sener A: Stimulus-secretion coupling of hypotonicity-induced insulin release in BRIN-BD11 cells. *Endocrine* 2006;30:353-363.

13 Sahara E, Rai T, Miyazaki J, Vekman AS, Sasaki S, Uchida S: Defective water and glycerol transport in the proximal tubules of AQP7 knockout mice. *Am J Physiol Renal Physiol* 2005;289:F1195-2000.

14 Bergmeyer HU, Berndt E: Determination with glucose oxidase and peroxidase; in Bergmeyer HU (ed): Methods of enzymatic analysis. New York, Academic Press, 1974, pp 1205-1215.

15 Leclercq-Meyer V, Marchand J, Woussen-Colle MC, Giroix M-H, Malaisse WJ: Multiple effects of leucine on glucagon, insulin and somatostatin secretion from the perfused rat pancreas. *Endocrinology* 1985;116:1168-1174.

16 Malaisse-Lagae F, Malaisse WJ: Insulin release by pancreatic islets; in Larner J, Pohl S (eds): Methods in Diabetes Research. New York, Wiley, 1984, vol 1, pp 174-152.

17 Miley HE, Sheader EA, Brown PD, Best L: Glucose-induced swelling in rat pancreatic islets. *J Physiol* 1997;504:191-198.

18 Best L: Glucose-induced electrical activity in rat pancreatic β -cells: dependence on intracellular chloride concentration. *J Physiol* 2005;568:137-144.

19 Best L, Brown PD: Studies of the mechanism of activation of the volume-regulated anion channel in rat pancreatic β cells. *J Membr Biol* 2009;230:83-91.

20 Majid A, Speake T, Best L, Brown PD: Expression of the Na-K-2Cl cotransporter in α and β cells isolated from the rat pancreas. *Pflugers Arch* 2001;442:570-576.

21 Hamann S, Herrera-Perez JJ, Zeuthen T, Alvarez-Leefmans FJ: Cotransport of water by the Na⁺-K⁺-2Cl cotransporter NKCC1 in mammalian epithelial cells. *J Physiol* 2010;588:4089-4101.

22 Panten U, Schwanstecher M, Schwanstecher C: Sulfonylurea receptors and mechanism of sulfonylure action. *Exp Clin Endocrinol Diabetes* 1996;104:1-9.

23 Best L, Davies S, Brown, PD: Tolbutamide potentiates the volume-regulated anion channel current in rat pancreatic β cells. *Diabetologia* 2004;47:1990-1997.